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Subcutaneous Dirofilariasis Caused by *Dirofilaria repens* Diagnosed by Histopathologic and Polymerase Chain Reaction Analysis

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SUMMARY A 31-year-old man with a history of intravenous drug abuse presented with an inflamed subcutaneous nodule in his left thigh. The nodule measured up to 1.2 cm in largest diameter. Under the clinical impression of an inflamed epidermal cyst or a subcutaneous abscess, surgical excision was performed. Histopathologic examination of the nodule and subsequent polymerase chain reaction (PCR) analysis revealed the presence of the helminth *Dirofilaria (D.) repens*, a member of the family *Filarioidea*. Dirofilariasis is a parasitosis caused by *D. repens* and *D. immitis* that most frequently affects canines. It can rarely be found in humans, usually in the form of a subcutaneous nodule. In Europe, most cases of human dirofilariasis have been detected in the Mediterranean countries, Ukraine and Russia, but sporadic cases have also been reported in central and north European countries. Dirofilariasis must be distinguished from other forms of filarial disease such as onchocercosis and *Wuchereria bancrofti filariasis*. Diagnosing dirofilariasis purely by histopathology has its pitfalls, especially when the morphology of the nematode is altered due to inflammatory response or surgical artifacts. PCR analysis offers an opportunity to confirm dirofilariasis and identify the dirofilarial species as well. Briefly, we conclude that a diagnosis of subcutaneous dirofilariasis should be considered in cases of subcutaneous mass in an endemic area of animal dirofilariasis.

KEY WORDS: dirofilariasis, helminth, subcutaneous nodule

INTRODUCTION

Dirofilariasis is an uncommon zoonosis caused by parasites of the genus *Dirofilaria*, most notably *Dirofilaria (D.) repens* and *D. immitis* in Europe (1). Canines represent the reservoir of infection, while human infections are sporadic. The infection is transmitted by the bite of a mosquito carrying parasitic larvae. In humans, the disease most often manifests as a subcutaneous nodule or, less

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frequently, as the so-called “coin lesion” in the lung comprising of microfilarial emboli in a branch of pulmonary artery (2). Cases of pulmonary dirofilariasis are usually attributed to *D. immitis* and subcutaneous nodules to *D. repens*; however, this does not comprise a strict rule, as there have been reports of both species causing pulmonary and subcutaneous infections (3,4).

We report on a case of dirofilariasis presenting as a subcutaneous nodule in the left thigh of a 31-year-old man from the Mediterranean part of Croatia, with a history of intravenous drug abuse. To our knowledge, this is the fourth case of dirofilariasis reported in Croatia and the first case confirmed by polymerase chain reaction (PCR) analysis.

CASE REPORT

A 31-year-old man with a history of intravenous drug abuse presented with an inflamed subcutaneous nodule in his left thigh. Physical examination showed solitary elastic to firm subcutaneous nodule, measuring 1.2 cm in largest diameter. Under clinical impression of an inflamed epidermal cyst or a subcutaneous abscess, surgical excision was performed. Histologic examination disclosed a dense dermal inflammatory infiltrate composed of eosinophilic granulocytes and histiocytes surrounding an adult helminth of the filaria species (Fig. 1A). The parasite was composed of a thick cuticle with longitudinal ridges (micro-ruffling of the surface), large lateral chords, tall, slender coelomian muscle layer, a single gut tube and spermatocytes in the genital tubule (Fig. 1B). The diagnosis of subcutaneous dirofilariasis caused by *D. repens* was established. On several sections, the helminth was found at the base of excision, so re-exsicion was recommended.

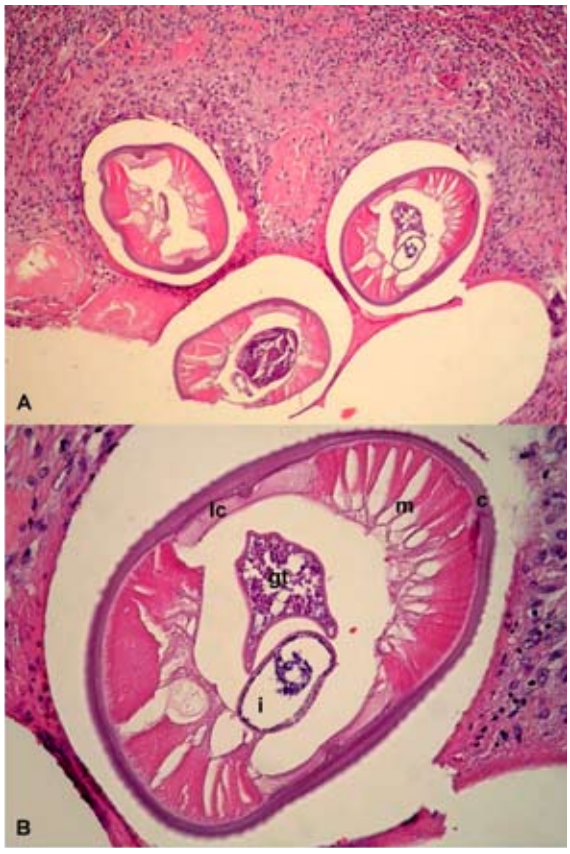

Figure 1. (A) Pronounced inflammatory reaction surrounding sections of the parasite (HE, 100x); (B) transverse sections of the parasite. All normal morphological aspects are clearly visible: cuticle with external ridges and micro-ruffling of the surface (c), myoid tissue fibers (m), lateral chords (lc), intestinal tubule (i), and male genital tubule containing spermatocytes (gt) (HE, 400x).

On second visit, a detailed history was available. The patient was originally from Dalmatia, temporarily working in Zagreb in an outdoor profession. The lesion had appeared 4 months prior to excision, which coincided with a history of the patient’s exposure to his employer’s dog. Re-excision was performed and revealed a nonspecific dermal inflammatory reaction with no remaining larvae.

Radiographic examination of the lungs performed to exclude lung involvement was unremarkable and showed no signs of coin lesion. Due to the fact that lesions may contain parasites of both sexes, a peripheral blood smear was performed prior to re-excision and did not reveal the presence of microfilariae. PCR analysis was performed to determine the species of *Dirofilaria*.

DNA extraction and polymerase chain reaction

Total DNA was isolated from archival formalin-fixed paraffin-embedded subcutaneous nodule section in duplicates. Paraffin was removed from the samples (20-50 mg) by serial washings in xylene (3 times) and absolute ethanol (6 times). The deparaffinized samples were dried and DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen S.p.a., Milan, Italy) according to the manufacturer’s instructions, with increasing digestion time up to 36 hours. PCR was performed with forward primer DIDR-F1 (5’-AGT GCG AAT TGC AGA CGC ATT GAG-3’) and reverse primer DIDR-R1 (5’-AGC GGG TAA TCA CGA CTG AGT TGA-3’), which amplify different fragment lengths of the internal transcribed spacer region 2 (ITS2) of the ribosomal DNA from *D. immitis, A. reconditum,*

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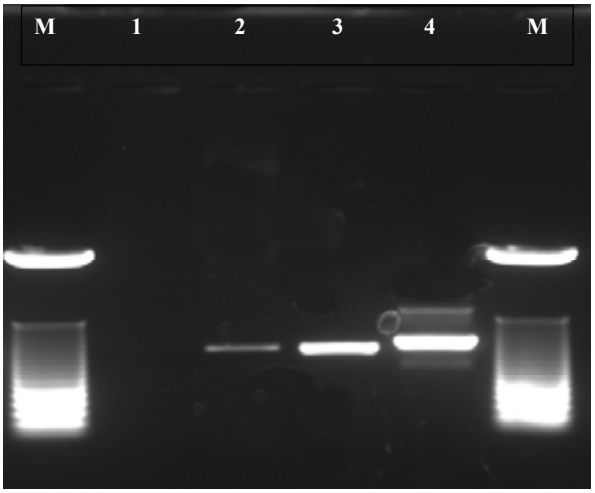

Figure 2. Gel electrophoresis of PCR products in 1.5% agarose gel. Lanes 1, 4: M marker (50 bp); lane 1: negative control; lane 2: DNA from archival formalin-fixed paraffin-embedded subcutaneous nodule; lane 3: reference *Dirofilaria repens* DNA; lane 4 reference *Dirofilaria immitis* DNA.

D. repens and several other filarial nematodes, according to Rishniw *et al*. (5). Each PCR reaction consisted of 1.5 mM $MgCl_2$, 10 mM dNTPs, 1.25 U of GOTaq polymerase (Promega) and 5 μL of DNA in a total volume of 50 μL. The PCR procedure consisted of denaturation step at 94 °C for 2 min and 35 cycles of denaturation (30s at 94 °C), annealing (30s at 60 °C) and extension (30s at 72 °C), together with final extension (7 min at 72 °C). The 10 μL PCR product was visualized on 1.5% agarose gel. The fragment characteristic of *D. repens* of around 484 base pairs was amplified in one sample (Fig. 2).

DISCUSSION

The majority of zoonotic filariae recovered from subcutaneous nodules in humans belong to the genus *Dirofilaria*. The species of *Dirofilaria* most commonly isolated from humans are *D. repens*, *D. immitis* and *D. tenuis*, while less frequent species include *D. ursi*, *D. subdermata* and *D. striata* (6). Filariasis caused by *D. repens*, which is the most common species in Europe, usually presents as a subcutaneous nodule, yet some members of this species have been found at the most various anatomic sites such as the epididymis, spermatic cord, lung, breast, omentum and subconjunctival tissue (7).

Various species of canines are the natural host of dirofilarias that reside in their subcutaneous tissue and produce microfilariae which circulate in the blood. The natural vectors are several species of mosquitoes, in which the development to third-stage larvae is completed.

Once dirofilarias are infested in humans as their final host, it takes several months for them to reach sexual maturity. In this stage, female worms can achieve maximum length of 17 cm and diameter of 650 μm, whereas males are smaller, about 7 cm by 450 μm^6. Subcutaneous nodules may contain one or paired adult worms of both sexes. In our case, we identified a male worm, which is a common finding in subcutaneous dirofilariasis (8,9). Even though *D. repens* microfilaremia in humans has, to our knowledge, been reported only once, peripheral blood smear was performed to exclude this possibility (10).

It is considered that countries of the Mediterranean basin, Ukraine and southern Russia are endemic regions of dirofilariasis in Europe; most cases have so far been described in Italy (1). There have been few reports from countries bordering to Croatia, i.e. two reports from Hungary, one from Serbia and Slovenia each. However, one of the Hungarian cases and the Slovenian case were considered to have been imported from Italy and Spain, respectively (11-14). To our knowledge, our case is the fourth reported case of dirofilariasis in Croatia; two of the former were observed in the Mediterranean part of Croatia and one in inland Croatia (15,16). All epidemiological data obtained from our patient indicated that he was infested in Croatia.

In most cases of subcutaneous dirofilariasis, diagnosis can be reached by biopsy. However, there are several factors that can cause problems in identification of parasite species; in settings where dirofilariasis is rare, the lack of familiarity with dirofilariasis and/or the parasite morphology may present a problem; in other instances, morphology of the nematode may be altered due to inflammatory response or surgical artifacts (17).

It is also very important to include other possible causes of filariasis in the differential diagnosis, such as bancroftian and malaian filariasis, onchocercosis, loaiasis and mansonellosis. These are infestations with *Wuchereria bancrofti*, *Brugia malayi*, *Onchocerca volvulus*, *Loa loa* and *Masonella perstans*, respectively (6). All of them may contain mature worms in subcutaneous lesions and they represent diseases that have more serious complications than dirofilariasis. Therefore, it is also essential to obtain appropriate epidemiologic data (2).

PCR genotyping is a valuable diagnostic tool that can help overcome all of the above mentioned obstacles; it is highly sensitive, even with minimal

amounts of parasite DNA, and highly specific, being able to make distinction between *D. repens* and *D. immitis* (5). This characteristic is not negligible, given the fact that *D. immitis* has a propensity to cause coin lesions in the lung (4).

In conclusion, there is evidence that Croatia, especially the Mediterranean part of it, represents an endemic area of dirofilariasis and the diagnosis of subcutaneous dirofilariasis should be considered on differential diagnosis of a subcutaneous mass. Physicians should be aware of this disease and its features for several reasons, primarily in order not to mistake coin lesions in the lung for malignancy and not to mistake dirofilarias in subcutaneous nodules for other, more harmful tropical parasites such as *Wuchereria bancrofti* or *Onchocerca volvulus*. In subcutaneous nodules, PCR analysis is an elegant way to overcome diagnostic obstacles caused by altered morphology and/or lack of familiarity with the specific morphology of filarial species. Surgical treatment of subcutaneous dirofilariasis in humans is considered curative and there is no need for further interventions besides clinical monitoring.

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